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Effect of Ammonium Chloride on Predatory Consumption Rates of Brook Trout (*Salvelinus fontinalis*) on Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) in Laboratory Streams

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Ammonium compounds are widely applied to the environment in the form of fertilizer, waste products, and fire retardants, sometimes resulting in their entry into freshwater streams. In aqueous media, an equilibrium between the ionized and un-ionized ammonium:ammonia forms develops. The un-ionized form (ammonia) is the principal toxicant to fish (CHAPMAN 1934). The proportion of the ammonium salt present as un-ionized ammonia varies primarily with pH and temperature (TRUSSELL 1972). Much is known about the chemistry of ammonia (NH_3) at low concentrations: it is highly water soluble, adsorbed by cation exchange sites, and readily converted to other forms of nitrogen. Ammonia is acutely toxic to various aquatic species at low concentrations (WILLINGHAM 1976, U.S. ENVIRONMENTAL PROTECTION AGENCY 1977). We are severely lacking, however, not only in our knowledge, but also in our methods for detecting sublethal effects of ammonia as well as most other environmental contaminants.

Exposing a fish to a sublethal level of a toxicant could modify predator-prey behavior leading to important changes in the population of valuable fish species. GOODYEAR (1972) has shown that exposure of mosquitofish to sublethal levels of ionizing radiation resulted in increased mortality when confronted by a predator. KANIA and O'HARA (1974) similarly demonstrated an impaired ability of mosquitofish to escape predation by largemouth bass after being exposed to mercury at sublethal concentrations. SULLIVAN et al. (1978), demonstrated an increase in the vulnerability of fathead

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minnows to predation by largemouth bass in laboratory aquaria due to exposure of the prey to sublethal concentrations of cadmium. A previous study from our laboratory (WOLTERING et al. 1978) indicated that sublethal effects of ammonia on predator-prey relationships could be detected using the largemouth bass (*Micropterus salmoides*) and the mosquitofish (*Gambusia affinis*), both warm water species. Ammonium chloride inhibited food consumption and growth rate of the bass.

The present study was undertaken to (1) devise an experimental apparatus to permit study of the impact of ammonia on predator-prey interactions involving salmonid fishes in a flowing water system and (2) quantify the effects of sublethal levels of ammonia in this system. This report describes the apparatus and the influence of ammonium chloride on consumption of salmon fry by trout in laboratory streams.

MATERIALS AND METHODS

The experimental apparatus consisted of 12 flowing-water artificial streams with a sand substrate. They were supplied with natural stream water with or without ammonium chloride. The streams were rectangular-shaped, 295- x 63-cm, containing a lengthwise partition which allowed the water to circulate. Each of the 12 streams had two test areas (Figure 1). Cover for the prey was provided by 10 large stones scattered throughout the length of each test area. A refugium in the form of a small mesh screen was built into the upstream end of each test area and provided a predator impenetrable sanctuary for the prey. Of the 12 streams, 3 served as controls, and 9 were supplied with toxicant; there were 3 streams at each of the toxicant concentrations, 1 stream with a prey density of 5, 1 with a prey density of 10, and 1 with a prey density of 15. The experiment was designed as a randomized block (two replications) factorial with prey density and concentration of ammonia as the factors.

Stream water velocity was obtained with a paddle wheel driven by a 12-r./min. gear motor and was held constant throughout the experiment. All streams were covered with translucent plastic to minimize the disturbance created by workers moving about in the laboratory and to keep the fish from jumping out of the stream.

Water containing various levels of ammonia as ammonium chloride was supplied by an 8-liter proportional diluter (MOUNT AND BRUNGS 1967) at the rate of appproximately 1 liter per minute per stream. The 95%

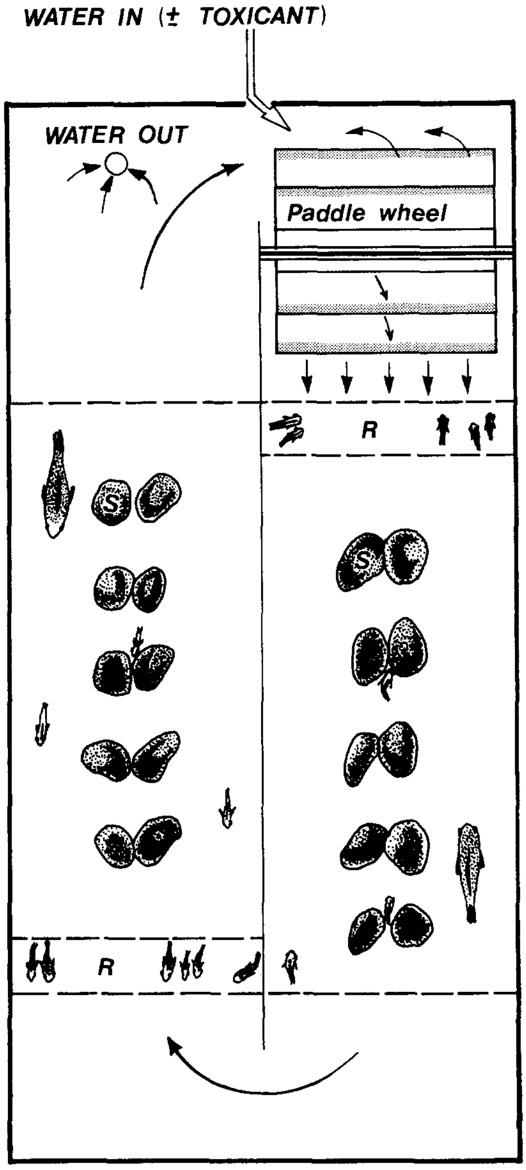

Fig. 1. Schematic diagram of a laboratory stream with two predator-prey interaction test areas. S = stone; R = refugium for prey.

replacement time was 18 hours (SPRAGUE 1973). Ammonium chloride was introduced into the diluting system by means of a Mariotte system and "dipping bird" similar to that described by MOUNT and BRUNGS (1967). Water samples from each stream were taken three times during the 7-day experiment, and total ammonium chloride was assayed using a specific ion (silver/sulfide) electrode (ORION RESEARCH INC. 1970). Concentrations ± standard deviation (± S.D.) of toxicant (as total ammonium chloride) averaged 3.9 (.52), 8.3 (.51), and 15.3 (1.1) mg/liter. We observed no mortality when the fish were exposed separately to these concentrations of ammonium chloride for 14 days. Based on water temperatures (range 9.4°-14.2°C, D.O. 7.4-8.1 mg/liter) and pH (8.0-9.7) at the time of sampling, the average un-ionized ammonia concentrations (± S.D.) were 0.29 (.08), 0.41 (.09), and 0.76 (.24) mg/liter, respectively, (calculated for each stream according to the methods of E.I.F.A.C. (1970)).

Each test area was stocked with one predator and either 5, 10, or 15 prey. The predators were adult brook trout (*Salvelinus fontinalis*) ranging from 129- to 221-g wet weight. The prey were juvenile chinook salmon (*Oncorhynchus tshawytscha*) ranging from 1.9- to 2.3-g wet weight. Brook trout were acclimated to the test areas and toxicant at least 7 days prior to the introduction of the prey and were deprived of food for 48 h immediately before the beginning of each experiment. The prey were acclimated to the appropriate level of toxicant for at least 4 days in 57-liter aquaria which received the effluent water from the stream into which they were to be stocked. Experiments began with the introduction of the prey into the interaction areas, and daily observations and counts were conducted throughout the 7-day test. Prey densities were maintained by daily restocking of acclimated salmon fry.

RESULTS

The artificial streams allowed both the predator and the prey to move about quite freely and provided an excellent system for behavioral studies. The cover provided by the rocks in midstream was sufficient to shelter the prey for short periods of time; but with active pursuit, the predator could force the prey into open water. The refugium at the upstream end of the test area provided an absolute sanctuary for the prey, and they discovered and utilized it almost immediately upon being introduced into the stream. The predator generally spent most of the daylight hours in the downstream third of the test area and the prey in the upstream half. In the streams with the higher toxicant concentrations, there was a more random distribution of prey throughout the test area.

Consumption rates of brook trout at all three densities of prey were very similar in control streams (Table 1). The lowest concentration of ammonia (0.29 mg/liter nonionized) caused consumption rates to drop as much as 65% below controls. As ammonia concentration increased, however, consumption of prey also increased. At the prey density of 5, consumption rates were close to control levels at the highest ammonia level (0.76 mg/liter) tested; at the prey density of 15, consumption rates increased above control levels as ammonia level increased. The consumption rate more than doubled (from 19.4 to 42.4 mg/g per day) at the highest ammonia level (0.76 mg/liter).

TABLE 1

Influence of prey density and ammonia on consumption rates of single brook trout preying on chinook salmon fry in laboratory streams

Prey[a] density	NH_3[b] conc. mg/l	Prey[c] consumed	Consumption[d] rate mg/g/day	Consumption rate % of control
5	0 (control)	8.3	19.2 (0.6)	100
5	0.29 (0.08)	3.8	6.75 (1.63)*	35.2
5	0.41 (0.09)	7.5	11.9 (6.6)	62.3
5	0.76 (0.24)	8.7	15.8 (4.3)	82.3
10	0 (control)	13.0	22.7 (5.0)	100
10	0.29 (0.08)	8.5	13.7 (2.2)*	60.1
10	0.41 (0.09)	7.3	12.3 (4.7)*	54.2
10	0.76 (0.24)	-	-	-
15	0 (control)	10.2	19.4 (8.9)	100
15	0.29 (0.08)	6.0	13.0 (1.3)	66.8
15	0.41 (0.09)	20.7	33.0 (10.0)	170
15	0.76 (0.24)	25.7	42.4 (8.7)*	218

[a]Salmon fry were restocked daily

[b]un-ionized ammonia ($\pm$ standard deviation)

[c]Numbers are averages of the total number of fish and consumed in the 7-day test period.

[d]mg of prey consumed/g of predator body weight/day ($\pm$ standard deviation)

*Significantly different from control (t-test) $P < 0.05$

These changes became more obvious when consumption rates were expressed as functions of prey density and ammonia concentration (Figure 2). When viewed in three-dimension, the graphical representation takes on a "butterfly" shape with the wings forming a V-shape which dips toward the common axis. Consumption rates at the lowest ammonia concentrations were below control levels regardless of prey density and described the vertex of the "V." At the two higher concentrations, consumption rates increased significantly with increasing prey density. (Note: due to an accident in one stream, there was no data gathered for prey density of 10 and ammonia concentration of 0.76 mg/liter.)

An analysis of variance revealed that increasing toxicant concentration and increasing prey density significantly increased consumption rates ($P < 0.01$). The interaction between toxicant concentration and prey density was also highly statistically significant ($P < 0.01$) indicating that the magnitude of the effect of ammonia is not the same at all prey densities.

DISCUSSION

Ammonium chloride had a profound influence on the predator-prey interaction studied in these experiments. The consumption rates of the predator brook trout were either depressed or elevated, depending on the concentration of NH_3 and the density of the prey (chinook salmon fry).

The toxicant had a greater effect on predator consumption rates at high prey densities than at low prey densities, illustrating the interaction between these two variables which was detected statistically. At the lowest prey density (5), consumption rates were depressed at all NH_3 levels tested. As the prey density increased, not only did consumption rates increase, but the effect of the toxicant became more pronounced. These results alone do not indicate whether the toxicant affected the predator in a positive manner or the prey in a negative manner, or both.

At the higher NH_3 concentrations, the salmon fry were observed to be sluggish and stuporous. Several mortalities which were not due to the predator occurred among the prey at the highest NH_3 levels. Our preliminary observation indicated that these levels of toxicant were not acutely toxic; thus it appears that the combination of the toxicant and the stress from the presence of the predator were synergistic. The brook trout exhibited toxic effects.

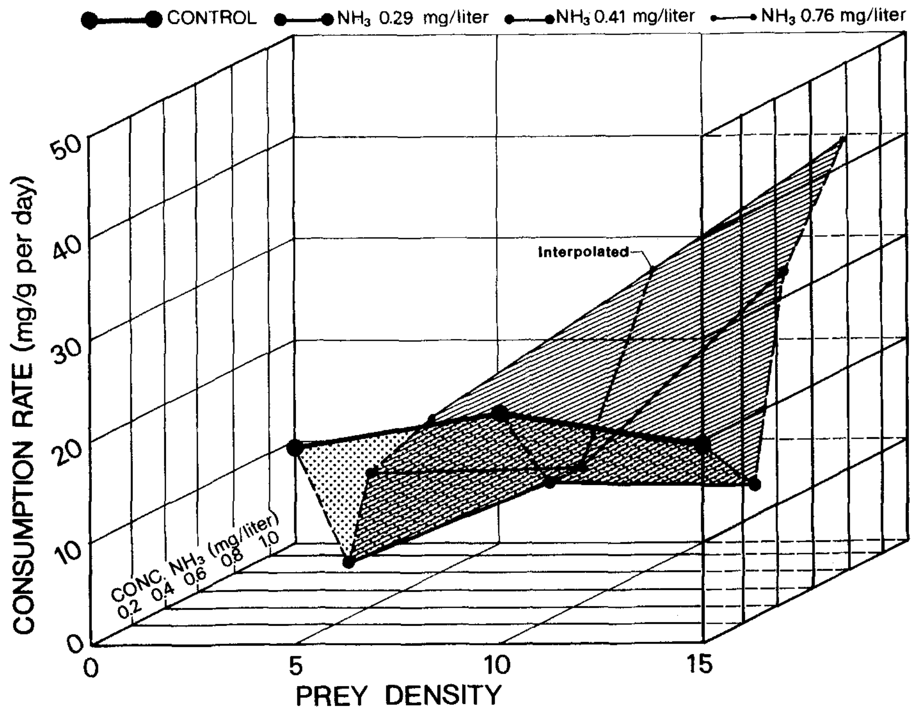

Fig. 2. Effect of prey density and ammonium chloride on consumption rates of predatory brook trout. Each point is the mean of three or four consumption rates. Broken lines connect data points from experiments with different concentrations of NH_3 but the same prey density. Solid lines connect data points from experiments with different prey densities but the same NH_3 concentration.

The prey in control streams spent most of their time either within the refugium or hiding among the rocks. Very little time was spent swimming freely. They also seemed to "sense" when the predator was feeding and rapidly moved to some cover. In the streams with high NH_3 concentrations, however, fry were frequently observed swimming freely or floating near the water surface. They appeared oblivious to the location or the feeding state of the predator. These observations suggest the toxicant was affecting the prey much more than the predator.

The reason for the interaction between prey density and ammonia concentration is not clear. Examination of the response surface (Figure 2) suggests that more than one mechanism was operating in the test system. At the lowest concentration, predation fell below control levels. Based on observation of activity, it appeared that the chinook fry were influenced by the toxicant to a greater degree than the brook trout. It is possible that the lowest level of ammonia tested decreased predation because of a stimulating effect on the prey which heightened their awareness of predator activity and their utilization of available cover. As the concentration of ammonia increased, the stimulatory advantage was lost, the prey became sluggish, and predation increased. Predatory activity increased at high prey densities, presumably due to the larger number of prey affected by the toxicant.

Our results and observations contrast sharply with those made by WOLTERING et al. (1978) in their static water tests with bass and mosquito fish. Their results indicated a greater influence of the ammonium chloride on the predator than on the prey. In the present study, the effect seemed to be exactly the opposite, i.e., the prey were more sensitive to the toxic effects of the ammonium chloride than the predator. It appears the behavioral effect observed is highly dependent, not only on the toxicant involved, but on the species of fish and the nature of the test system as well.

The test apparatus used in this experiment was useful in evaluating the sublethal effects of ammonia on predator-prey relationships among two species of salmonids. Other types of organism responses like territorial responses, courtship or breeding responses, and food utilization are examples of effects which may be influenced by sublethal exposure to environmental contaminants and which could be tested in this type of system. In our experiment, ammonium chloride did modify the baseline predator-prey interaction as measured by changes in predator consumption rates.

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